Waters Lab Highlights

An Internal Communication of Applications and Techniques

LAH 0432 6/90
AN/PA/RS/DR/AV

# Analysis of Carbamazepine by Liquid Chromatography with Surfactant Containing Eluents: I. Direct Injection of Serum

LC is currently used for the analysis of a wide range of drugs from serum samples in clinical, pharmacokinetic and pharmaceutical research laboratories. In most cases, it has been necessary to clean up the serum sample prior to injection in order to remove proteins and other serum components which might bind to the column packing or otherwise interfere with the analysis. Solid phase extraction procedures, executed manually or via a MilliLab™ Workstation, play an important role in such cleanup steps, particularly for drugs present at low levels in serum.

Recently, a number of approaches which allow the direct injection of untreated serum have been reported. In some cases, a specially designed packing material has been used. A more generally applicable approach uses conventional reverse phase packing materials in combination with eluents containing surfactants such as sodium dodecylsulfate (SDS), Brij or CHAPS[1]. Literature reports to date have described micellar eluents, in which the surfactant is present at a concentration high enough to form micellar aggregates of surfactant molecules. However, work at Waters[2] indicates that the presence of micelles is not critical, as long as serum components are adequately solubilized. Thus, surfactant containing eluents provide a broad range of options for the analysis of drugs with

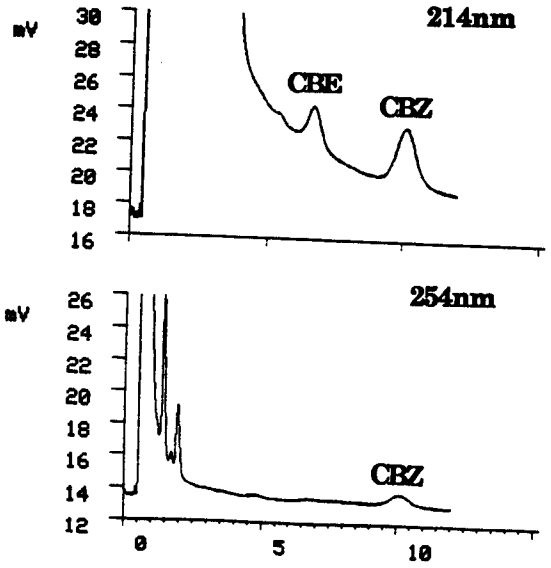


**Figure 1. Separation of Carbamazepine (CBZ) and metabolite (CBE) from spiked bovine serum with UV detection at two wavelengths. See Table 1 for conditions. Note that detection at 254nm is unsuitable for this application (see text).**

Waters
Division of MILLIPORE 34 Maple Street Milford, MA 01757 508-478-2000

Vince Warren/Stefan Schmitz

direct injection of serum, including the control of selectivity and retention through the manipulation of organic solvent blends, pH, ionic strength and other eluent variables much as in paired ion chromatography.

The analysis of carbamazepine and its epoxide metabolite is often done by LC[3] due to the need to monitor the carbamazepine epoxide metabolite in addition to the parent compound, a task which currently available immunoassay approaches cannot perform. The therapeutic levels of carbamazepine are relatively high (4-10 µg/mL serum), and the parent compound itself is readily detected at 254 nm or higher in the UV. The epoxide metabolite, however, must be detected at a lower wavelength (Figure 1) which accentuates the tailing of the "serum peak."

Using the conditions summarized in Table 1, we have successfully run more than 800 sequential injections (10 µL) of bovine serum spiked with clinical levels of carbamazepine and its epoxide metabolite. The only precautions required were: 1) refrigeration of the serum sample within the autoinjector or use of the serum sample within 2 hours at room temperature and 2) change of the Guard-Pak™ cartridge and in-line filter after each 50 injections. Reproducibility of the retention time for each analyte and was 3% or better (relative standard deviation) for the entire set of more than 800 injections. For peak areas, precision was 8-10%. Some of the variability in peak area may be due to the difficulty of integrating peak areas on the tail of the serum peak. To improve upon this situation, we have investigated a column switching approach which passes the serum peak to waste followed by a backflush of the analytes onto an analytical column for analysis on a flat baseline.

Overall, it appears that surfactant containing eluents can be used with conventional reverse phase columns to give hundreds of successful injections of serum with good reproducibility of retention times. During the past year, we have made thousands of spiked serum injections using a variety of surfactant containing eluents, and have typically achieved column lifetimes of 700-1000 injections. Direct serum injection will be most satisfactory when the detector can be adjusted to give a minimal signal for the serum peak (e.g. fluorescence or UV above 300nm) while maintaining a strong response for the drug(s) of interest.

**Table 1**

| | |
|---|---|
| Column: | µBondapak™ Phenyl (0.39 x 15 cm, steel) |
| Guard-Pak Cartridge: | µBondapak Phenyl |
| Eluent: | Methanol/water with 50 mM SDS (30/70) |
| Injection: | 10 µL of spiked bovine serum from refrigerated WISP 712 |
| Drugs: | Carbamazepine (CBZ) at 4.8 µg/mL serum<br>Carbamazepine-10,11-epoxide (CBE) at 2.0 µg/mL serum |

References:


1. D. Westerlund, *Chromatographia*, **24** (1987) 155.
2. U.S. Patent application #07/364,057 (June 1989).
3. LAH 0424 3/90.


* * *